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MODE OF ACTION OF SOME TOXIC SUBSTANCES WITH SPECIAL REFERENCE TO THE EFFECTS OF PROLONGED EXPOSURE*

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In the first Ernestine Henry lecture, entitled "Devices to Protect the Worker," Dr. Donald Hunter said: "Medical examination with adequate laboratory aids should detect intoxication before disability occurs." If it be permitted to include studies in the experimental laboratory among the "aids" referred to by Dr. Hunter then what follows may be regarded as coming within the terms upon which this lectureship was established.

In many occupations, both old and new, men and women are brought into contact with all kinds of materials which in one way or another are capable of behaving as poisons. As technology advances not only certain groups of industrial workers but also the general public meet with an increasing number of chemical substances, some of which may have only recently been created. Past experience, however, has indicated that it may take many years before the incidence of a disease is recognized as having a relation to exposure to a chemical. It is therefore not surprising that voices have been raised in alarm at the possible threat to health that may be associated with prolonged exposure to new substances. It would probably be fair to say that much more alarm has usually been expressed on behalf of the public who may come in contact with traces of a new material than on behalf of the smaller groups who may, by their occupations, come into much more intimate contact with much greater amounts. In this context it is worth bearing in mind the general epidemiological principle that the larger the dose the more likely is an effect to be seen.

If it is suspected that a chemical might have a delayed toxic effect or an effect that is only obvious after prolonged exposure to the material, a search for any reaction should begin among the appropriate sections of the population. For example, alarm is not infrequently expressed that the traces of some of the new insecticides which may find their way into our food could eventually prove injurious to the health of the consumer in a manner usually not specified. Some years ago the World Health Organization tried to find out from several large manufacturers what, if any, experience of illness they had had among those who made such insecticides. The replies made it clear that the large responsible manufacturer of a poisonous new product protects his employees from any significant exposure to it. It therefore seemed unlikely that much would be learned from a long-sustained study of such small specialized working populations.

As in the case of many other new chemicals, the people who are more likely to be exposed seriously to

new insecticides are those who use them. Clearly it is going to be very much more difficult to conduct an epidemiological surveillance lasting many years over groups of local labourers in some tropical country who are exposed to an insecticide year after year in the course of a malaria eradication campaign, or to small groups of men in a number of engineering shops heavily exposed to some new degreasing solvent. The value of proper studies on man in order to gain knowledge of the potential hazards which poisonous materials may present to him cannot be overestimated. However, the difficulties and duration of any searches for the effects of prolonged exposure underline the need for, and indeed the desirability of, discovering other criteria that may enable one to predict with reasonable certainty whether or not a new chemical is likely to prove dangerous to man.

The alternative criteria which first spring to mind are those that can be obtained from studies of the effect of a chemical on biological systems other than man himself. An attempt will be made to consider this problem in a general way with a few illustrations from among many substances that may present a hazard to those who make or use them. It is important to try to understand how far studies in the experimental laboratory may make it possible to draw reasonable conclusions about the probable effects of new substances upon man. Particular consideration will be given to the possible effects from prolonged exposure because from every point of view these are the most difficult to determine.

Maximum Allowable Concentrations

A poisonous substance given either as a single dose or in repeated doses over a short period produces a distinct and recognizable effect upon function and structure. Where prolonged exposure has to be incurred the practice of good industrial hygiene will create conditions in which the amounts absorbed by the people using the toxic product will not produce the signs and symptoms of poisoning characteristic for that material when received in larger amounts over a shorter period. Estimates of what are thought to be innocuous dose levels to which it may be safe to allow exposure for limited periods, usually eight hours daily for five days a week, are embodied in the figures for maximum allowable concentrations (M.A.C.). These, of course, apply only to those substances that are absorbed from the working atmosphere via the respiratory route.

By analysis of workshop air it is possible to get some idea to what a group is exposed, though this may provide

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only a very rough approximation to the actual exposure where many different operations are carried out in a workshop. It is not normally possible to estimate the dose received by the percutaneous route of absorption. Special studies of this kind have been made in certain operations, such as those in which insecticide sprays have been applied to large numbers of houses in disease eradication programmes. Despite the apparent hazard from the inhalation of the spray droplets in these confined spaces the contamination of the exposed skin by droplets was found to be 200 times as great as from that which was inhaled (Wolfe *et al.*, 1959). Many solids as well as liquids can penetrate the intact skin, so that it is necessary to bear in mind that the factory air may only be one of the routes of absorption. The dose of chemical a man actually receives during a working day may be far greater than that which he actually inhales. The importance of skin absorption and ingestion will naturally vary greatly with the type of operation and the extent to which the worker is physically contaminated by the substances.

Figures for the M.A.C. of gases, fumes, and dusts that may present a hazard in industry are issued by the United States Industrial Hygiene Association and have been available for years. These figures have recently been adopted by the Factory Inspectorate in this country, and very similar figures are used in several European countries. A set of figures in which the safe levels of many of the same materials are very much smaller is issued by the U.S.S.R. (Elkins, 1961). The reason for the discrepancy between these recommended levels is discussed later, but it is as well to note that these higher standards are apparently to be applied only to new factories within the U.S.S.R. The general population may come into contact with other substances, such as many of those now added to manufactured and processed food, many of which have no demonstrable toxic effect when given in single doses. This does not prevent some people from expressing alarm that ill effects may eventually appear if small quantities of such materials are taken over very long periods.

Study of Toxic Action

For the experimental toxicologist to be able to provide answers to the questions concerning the quantity of a poison that may be ingested safely for an indefinite period, he needs to undertake a detailed analysis of the mode of action of the poison. To satisfy some of those who worry whether an inert material will remain harmless however long it is ingested may be psychologically impossible. To assure himself, however, that the substance is truly inert in the doses envisaged calls again for a study of its behaviour in the animal body so that the reason why it does not harm the organism can be explained in scientifically satisfactory terms.

How can a study of the toxic actions of certain substances be interpreted in such a way as to act as a guide for defining probabilities about the effects on man from a prolonged exposure? The most obvious place to begin is the point of contact between the animal and the chemical. However, despite its tremendous importance in occupational medicine, damage to the skin will not be considered here. Local effects on the mouth or alimentary tract do not loom large in this field, and, like the eyes, these seem to be sensitive areas where unpleasant contact is readily noticed and avoided. The lower respiratory tract appears, however, to be

different. There is much interest in the field of atmospheric pollution and its possible relation to disease, especially in the elderly and longest exposed.

Irritant Gases

Sulphur dioxide is one of the irritant gases that have been studied in the laboratory. The M.A.C. for industrial exposure is 5 p.p.m. Amdur *et al.* (1953) claimed that an exposure to 1–8 p.p.m. for only 30 minutes could produce changes in the respiratory pattern of healthy human subjects, but Lawther (1955) was unable to repeat their observations. However, Amdur (1958–9) has found that 2.6 p.p.m. for an hour will increase the airway resistance of the guinea-pig. Dalhamn (1956) has described some interesting changes in the bronchial mucosa of rats exposed to 10 p.p.m. of sulphur dioxide for 10 weeks. Both the quantity and quality of the mucous secretions changed and there was multiplication of the mucus-secreting cells in the depth of the bronchial epithelium. These changes had regressed little, if at all, in animals examined four weeks after exposure to sulphur dioxide had ceased. One characteristic feature of the bronchial mucosa in chronic bronchitis in man is an increase in the numbers of mucus-secreting glands and goblet cells (Reid, 1960). Despite the changes wrought in the mucosa of these rats the cilia, though less effective in moving the altered mucus, remained as lively as ever, beating 1,200–1,300 times a minute.

These interesting studies seem not to have been extended, despite the indications they provided of the action of an irritant gas on the respiratory mucosa. Other poisonous gases react first with the alveolar lining of the lungs, and over-exposure results in pulmonary oedema. A method has been described by Long and Hatch (1961) in which the efficiency of the absorption of ^{14}C -labelled carbon monoxide through the lungs can be studied in intact animals. It is perhaps rather disquieting to note that an effect on the absorption of carbon monoxide, which probably reflects some change in the alveolar lining of the lungs, could be detected in rats which had been exposed for only 30 minutes to 1 p.p.m. of phosgene. This is the concentration which is thought to be safe in a working environment. If man is as sensitive as the rat to phosgene, such a concentration can hardly be called an ineffective level. With this new technique the effects of other irritant gases against more species could be studied, and these first observations indicate an urgent need for more work on these lines to be undertaken.

Pattle (1958) described an interesting material which coated the bubbles expressed from mammalian lung. He deduced that the presence of this material as a monomolecular layer prevented mammals from drowning in their plasma abstracted from the blood by the capillary attraction exerted by the small air-filled alveolar spaces. That this lipoprotein (Pattle and Thomas, 1961) has the exact role in the lung which Pattle assigned it has been questioned (Mead, 1961). Nevertheless the action of lung irritants upon its production and behaviour seems worthy of further study, particularly in relation to the effects of repeated exposure. For example, it has been shown that a small dose of ozone will protect mice for many days from the pulmonary oedema that results from exposure to higher concentrations of this gas (Stockinger *et al.*, 1956; Matzen, 1957).

For a country in which respiratory disease takes such an annual toll in death and disability perhaps more could be done to study the intimate reactions between toxic and irritating materials on the delicate and vital structures of the respiratory tract. No one is certainly in a position to be anything but cautious and pessimistic in predicting the likely effects of prolonged exposure to toxic materials which act as primary irritants on the lungs.

The effects of substances after they have been absorbed will now be considered. Before leaving the lungs the problems presented by dust are briefly discussed. Any particles in the inhaled air which are below a certain size—about 7 microns of unit density—will reach the lungs. Unless they are very small—less than 0.1 micron—most of these particles will be retained. A mechanism for clearing the lungs of inhaled particles exists, but the colour of the lungs of any healthy middle-aged city dweller demonstrates that this mechanism is inadequate to keep the lungs free of inhaled soot even for those who live in what might be regarded as a normal environment. Whenever dust is inhaled in excessive quantities it can be assumed that substantial amounts will be retained within the phagocytic cells in the pulmonary tissues.

The Pneumoconioses

Of the pneumoconioses, silicosis is the best known, the most widespread, and probably the most serious. Simple experiments indicate that silica has a toxic effect upon cells anywhere in the body, so it is not surprising that serious tissue injury develops round deposited silica. However, there is no simple explanation of why silica is toxic or why silicosis is such a serious disease. It is by no means easy to follow the various arguments for and against the solubility theory of silicosis (Holt, 1957). Alternative and complex hypotheses presumably reflect some difficulty in accepting the idea that silica particles are toxic because the toxic silicon dioxide gradually leaches out of them into cells containing or surrounding the particles. It has been suggested that silicosis is a disease in which the development of some degree of auto-immunity plays a part (Vigliani and Pernis, 1959). The antigen suggested is a polysaccharide believed to be liberated from macrophages killed by the silica which they have ingested. If this notion of auto-immunity comes to gain general acceptance as an explanation of the lesions of the pneumoconioses, the present state of knowledge will not permit very much use being made of animal experiments for the purpose of predicting whether diseases of this type will follow the inhalation of a new dust.

Indeed, the limitation of animal studies in this connexion had already been made obvious by the problems presented by berylliosis. The serious nature of the chronic and progressive form of this disease in man was recognized soon after the second world war. As an occupational disease it disobeys some of the usual rules, particularly the one in which the severity of the disease is related to the extent of exposure to the noxious material. While there is no doubt about the toxicity of the beryllium ion, and while there is some histochemical evidence that beryllium ions leach into the tissues from deposits of insoluble beryllium oxide, there seems equally little doubt that the element of hypersensitivity plays an important part in affecting the course of the disease in some, if not all, the victims (Sterner and Eisenbud, 1951). Nevertheless, it has

proved impossible to demonstrate hypersensitivity to beryllium or silica in guinea-pigs using techniques that are effective with other materials (Collet *et al.*, 1961). If auto-immune phenomena play a part in both silicosis and berylliosis the precise mechanism must be quite different if one may judge by epidemiological experience of the two diseases, and it would seem quite misleading to group the conditions together (Gross, 1960).

When a completely inert dust is inhaled in large quantities dramatic changes in the radiograph of the chest may be seen with a heavy element like stannous oxide, yet no disease results (Robertson *et al.*, 1961). This might be predicted from the complete failure to show that stannous oxide has any action whatsoever on biological tissues. Nor does there seem to be any evidence that the presence of so much inert material in the lymphatics and macrophages of the lung will adversely affect its response to infection or other added burdens.

Asbestos, on the other hand, not only produces a severe pneumoconiosis but is believed to be responsible for a high incidence of two distinct malignant lesions—bronchial carcinoma (Doll, 1955) and pleural endothelioma (Wagner *et al.*, 1960). This dust seems to be one that urgently requires further experimental study. Although carcinoma of the bronchus is in the forefront of the clinical problems of this age, the condition is not readily reproducible in animals. Radioactive pellets implanted in the bronchus will produce typical squamous epitheliomata in rats (Nelson, 1961), and a single example of this lesion from diazomethane has been recorded (Schoental, 1960). No explanation can yet be offered for the effects on the lungs of the prolonged exposure to cigarette tobacco smoke.

Delayed Effects

Where a substance enters the general circulation through the lungs, skin, or alimentary tract the likelihood of a prolonged administration producing delayed effects will depend upon a great many different factors. A very important one will be whether the excretion of the toxic material or the products of its breakdown is maintained. Despite the fact that the majority of poisonous or foreign materials that are ingested will eventually be excreted through the kidney, this rarely seems to be the site where the long-term effects of poisons are manifest. The kidney is extremely sensitive to acute damage by single doses of a common poison like mercury. The precise point of binding of mercury with some elements of the renal tubule has been studied in animals (Surtshin and Yagi, 1958). When mercury salts were fed over long periods the only lesions seen in laboratory animals were in the kidneys (Fitzhugh *et al.*, 1950), yet men exposed to mercury may continue to excrete mercury in large quantities without developing any manifestation of renal failure. Instead, if exposure to mercury is excessive the first signs of poisoning in man are disturbances of function in the nervous system.

It is to be expected that any impairment of the function in the central nervous system would be more readily recognizable in man than the earliest signs of renal damage. However, mercury-poisoning does provide an example of a common poison about which it is possible that more might be learnt from experimental toxicology if methods of studying the function of the nervous system were better developed. That kidney injury does not appear commonly as an effect

of a prolonged exposure to poisonous substances may partly explain the comparative lack of interest shown by experimental toxicologists in this important organ.

Before many chemicals—particularly the less water-soluble organic molecules—can be excreted by the kidney they must be rendered water-soluble; otherwise they pass through the glomerulus and are promptly reabsorbed by the tubules. This step of making a substance more water-soluble is probably one of the most important reactions, if not the most important one, the body shows to the introduction of a foreign chemical. This concept owes much to the work of Brodie *et al.* (1958) in the United States. The cellular components responsible for these metabolic transformations can be isolated, and their behaviour has been studied in the test-tube. Work has hitherto been largely devoted to an elucidation of the various chemical changes, such as oxidation, deamination, dealkylation, or hydroxylation, which compounds of different chemical structure may undergo, and to the enzymes and co-factors that bring about these changes. Recently attention has begun to be paid to conditions which may disturb these important activities. Thus cell fractions isolated from the liver of a rat that has fasted less than 24 hours may be quite unable to perform a reaction readily accomplished by the same fractions from a fed rat (Roth and Bukovsky, 1961). Cell fractions from the liver of a jaundiced rabbit also perform very poorly compared with those from a healthy animal (McLuen and Fouts, 1961). A study of these activities and their significance in the reaction of the intact animal to poisons is, however, only yet at an early stage.

Molecules can also be made more water-soluble by conjugation. A well-known step of this type is a reaction with glucuronic acid, brought about by the enzyme uridine diphosphate glucuronic acid transferase. This enzyme has been intensively studied, for it is in constant operation conjugating bilirubin and sex hormones. Chemicals that the body cannot use, some of which may even be toxic, are continually being absorbed as minor constituents of natural foodstuffs or as the products of bacterial action in the gut. The materials for conjugation, such as glucuronic acid and glycine, are freely available. Drugs, industrial chemicals, or non-nutritive substances added to food will be dealt with by these enzyme systems provided that the foreign chemicals are not so toxic that they injure the liver cells and interfere with these detoxication processes. Thus for a non-toxic material such as benzoic acid that is conjugated and completely excreted there would seem to be no reason why this process should not continue indefinitely. The ingestion of such material over long periods would not therefore be expected to have any untoward effect.

Conjugation and Excretion

For many of the simpler aromatic hydrocarbons which are used widely in industry, conjugation and excretion are remarkably efficient. With the exception of benzene these hydrocarbons have not proved dangerous except in very high concentrations; then they exert acute effects, usually on the central nervous system. The usual detoxication process is one of side-chain oxidation, followed by conjugation and then excretion. Benzene, having no side-chain, undergoes a complex series of oxidative reactions which, for at least some of it, includes breaking of the ring (Williams, 1959). Benzene, but none of its closely related homologues, has a

profound toxic action on the bone-marrow of man, and this can also be demonstrated in various laboratory animals. Gerarde (1960) believes that it may be the products of the oxidation of benzene that are responsible for this action, though he stresses that there is no positive evidence for this. Its power to damage the bone-marrow is a good reason for treating benzene as a very poisonous material, and it is not surprising that as observations on factory workers exposed to benzene have improved in scope and duration the M.A.C. has been progressively lowered.

Apart from the case of benzene, myelotoxic injury seems to be a problem more often met with in therapeutics than as a sequel to exposure to industrial chemicals. It is never possible to be certain that a chemical will not affect some people in this way just because myelotoxic effects were not produced in laboratory animals. Recent work on the haemolytic action of a number of chemicals has shown that certain individuals are unduly sensitive because their red blood cells lack an enzyme glucose-6-phosphate dehydrogenase (Carson *et al.*, 1956). This enzyme is required to maintain adequate levels of reduced glutathione in the red cell (Beutler, 1957). Lowering the level of glutathione makes the red cell, particularly the older cells, more sensitive to haemolysis and it also predisposes to the formation of the blue inclusion bodies called Heinz bodies (Beutler *et al.*, 1955). These red-cell inclusions have been observed among industrial workers exposed to many different aromatic compounds.

The list given in the review by Buckell and Richardson (1950) may be compared with a recent list of chemicals, including some natural products, that produce haemolytic anaemia in sensitive subjects (Larizza *et al.*, 1960). This inborn defect is more common in certain races and may possibly have accounted for the undue sensitivity of four negro children to naphthalene reported some years ago (Zuelzer and Apt, 1949). When the chemical industry spreads to Africa, medical officers may find problems not yet encountered in Europe and North America. This illustration of the role which genetic make-up may play in determining the sensitivity of individuals to chemicals might suggest that a similar type of defect could be related to the sporadic incidence of agranulocytosis among those receiving certain drugs. It also provides an argument against the use of pure-line animal strains in any laboratory screening tests for the toxicity of new materials.

Returning to the metabolism of foreign compounds, it may happen that in making fat-soluble molecules more water-soluble the liver produces a more toxic metabolite. One example from among well-known industrial poisons is the case of tetraethyl lead. This substance is biochemically inert but also insoluble in water, so that if it is accidentally inhaled it must be made water-soluble before it can be excreted. Tetraethyl lead does not concentrate in the brain as is sometimes supposed but is metabolized in the liver, where one of the ethyl groups is removed to form the water-soluble triethyl lead ion (Cremer, 1959). This gets into the circulation, and by its interference with cellular metabolism in the brain it produces a serious and often fatal poisoning. Anyone who began by studying tetraethyl lead in isolated biochemical systems would deduce that it was inert; however, triethyl lead salts are very toxic. Attempts to understand the mode of action of toxic substances must include *in vivo* and *in vitro* studies in order that a complete picture may be obtained.

Trichloroethylene

The metabolic transformation undergone by a chemical may be very complex and have a bearing in a number of ways on assessing its safety. Trichloroethylene is a very common industrial solvent which has also been used as an anaesthetic. It is generally accepted as being a substance of low acute toxicity, but its widespread use has aroused much interest in its possible long-term effects on man. As a result of numerous studies on animals and on man it is generally agreed that trichloroethylene undergoes a series of metabolic changes soon after it is absorbed. It is probably converted first to chloral hydrate, and this is then oxidized partly to trichloroacetic acid and partly to trichloroethanol. The latter is pharmacologically active, but it is conjugated and rapidly excreted as the glucuronide (Butler, 1949). The M.A.C. for trichloroethylene in various countries ranges from 200 p.p.m. to 9 p.p.m. Swiss and Swedish workers have made extensive studies in factories where trichloroethylene has been used as a degreasing agent (Grandjean *et al.*, 1955; Andersson, 1957). The concentration of trichloroethylene in the factory atmospheres has been compared with the 24-hour excretion of trichloroacetic acid in the urine of the exposed workers. (Ahlmarm and Forsmann, 1951; Grandjean *et al.*, 1955).

The important feature of both the Swiss and the Swedish reports is the emphasis placed upon the incidence of subjective symptoms in the workers exposed to trichloroethylene. Although the workers studied in Switzerland were described as being of a very poor general standard, far below that of the average Swiss artisan, the investigators apparently found it possible to give weight to conditions described as "emotional lability," "listlessness," "fatigue," and other similar complaints. The Swedish observers claimed that there was an unusually high incidence of subjective complaints, which included a reduced tolerance to alcohol in people excreting 40 mg. or more of trichloroacetic acid per litre. It is on the basis of an alleged safe level of trichloroacetic acid excretion (20 mg./l.) that the Russian M.A.C. of 9 p.p.m. appears to have been set (Grandjean *et al.*, 1955). The low Russian M.A.C.s for many common industrial chemicals are apparently derived from studies of the effects of these compounds upon "higher nervous activity."

Effects on the C.N.S.

All toxicity-testing procedures on laboratory animals include an examination of the effects of the compounds upon conditioned reflexes, which are rather widely defined. Only atmospheric levels of a vapour that are below those which can produce changes in the behaviour of laboratory animals are regarded as safe for man. While it is not difficult to understand how a substance like trichloroethylene, or carbon disulphide, could have effects upon the central nervous system which are first detectable only by sensitive behaviour studies, it is hard to understand why sulphur dioxide, which is very unlikely ever to reach the brain when inhaled in low concentrations, can have such an effect. It seems possible that some of the modifications in the behaviour of these test animals may not necessarily reflect an adverse reaction but indicate merely that the animal can still smell the substance.

This whole subject has not yet been adequately explored either in the experimental laboratory or in the

industrial medical officer's clinic. Probably no one is in a position to say what importance the long-term ingestion of materials at levels liable to affect higher nervous activity will have in relation to occupational disease, either organic or subjective. As standards of industrial hygiene advance it is to be expected that more emphasis will be given to complaints of sleeplessness, fatigue, or irritability as possible reflections of some toxic effects.

While it is obviously difficult to try to decide what will be a safe dose of a substance where the earliest detectable effects are subjective changes such as those affecting behaviour, the position may not necessarily be any easier in the case of the more specific biochemical reactions to toxic substances. Blood or urine levels of toxic materials have been accepted in some but by no means all instances as criteria upon which to base decisions on the safety or otherwise of the degree to which exposure appears to be taking place. It seems probable that measurements of more specific biochemical changes will in the future be more frequently included as measures of exposure.

Already blood cholinesterase levels provide a valuable guide in detecting exposure to the widely used organophosphorus insecticides. Man is richly endowed with this enzyme in his blood-stream; indeed, it is not yet known why he has so much, since he can apparently manage perfectly well if the activity of this enzyme is slowly reduced to 1/10 of that which Nature gave him. If this normal level is depleted, say, by 20%, as might happen over fairly long periods among those spraying insecticides in public-health programmes, should this be considered to be harmful? There is no evidence that laboratory animals suffer in any way from a lifelong partial depletion of their blood cholinesterase. However, if their brain enzyme is depleted by 50% or more, which occurs only after an even more severe depression of the blood enzymes, it is possible to detect slight aberrations in the behaviour of trained rats (Russell *et al.*, 1961). In the present state of ignorance it is probably justifiable to say that any prolonged inhibition of blood cholinesterase in man is undesirable despite a lack of evidence that it is injurious.

A somewhat different attitude seems to be taken with respect to carbon monoxide—a very common hazard both in industry and in ordinary life. There has long been a controversy on whether such a condition as chronic carbon monoxide poisoning really exists. One monograph which stakes a claim for the existence of the condition lists disorders of practically every system as part of the syndrome (Grut, 1949). Judged by modern textbooks on industrial disease and a recent detailed study (Lindgren, 1961), the consensus of opinion is that there is no such condition as chronic carbon monoxide poisoning. Now, carbon monoxide reacts with another element present in relative abundance in our blood, and it seems to be generally accepted that the presence of 5% COHb in the blood is of no consequence. Nevertheless it was possible to demonstrate a slight effect on the visual acuity of healthy young men by inducing this degree of carbon monoxide saturation of haemoglobin (Macfarland *et al.*, 1944). No one has yet established a link between persistent degrees of carbon monoxide saturation of the blood and human disease. Perhaps this provides support for the idea that the human body can tolerate a certain minor degree of injury for long periods without serious consequences suddenly making their appearance. The situation with regard to carbon

monoxide also illustrates the point that well-known poisons seem to excite less anxiety than new chemicals, whether poisonous or not.

Poisoning after prolonged exposure to a toxic material may result from the fact that the substance has accumulated or remained for long periods in the body. Arsenic in small quantities may collect in the hair and nails and do little damage, but lead collects in the bones and the first toxic effects are seen as disorders of haemoglobin metabolism in the adjoining bone-marrow. The effects of radium on bone are also well known.

Another tissue in which accumulation can take place is fat. The importance of fat as a reservoir for surplus material circulating in the blood is only now coming to be recognized. The short-acting barbiturates owe their effects to the fact that after an intravenous injection the blood level rapidly falls below an anaesthetic level as the compound disappears into the fat (Brodie *et al.*, 1950). As the blood level falls as a result of metabolism of the barbiturate in the liver the surplus is then slowly released from the fat into the blood, but in sub-anaesthetic levels. The same course of events probably occurs in trichloroethylene anaesthesia. In the case of carbon tetrachloride it has been shown that by far the greatest fraction of a single dose goes into the fat (McCollister *et al.*, 1951). It seems very probable that the hangover from acute poisoning by many highly fat-soluble materials may be due to this, and any so-called safe level of exposure to such materials should only be to doses that will not lead to a progressive accumulation of the compound in fat.

The fat itself seems to serve as a passive receptacle. Thus D.D.T. is readily laid down in the fat of rats which receive D.D.T. in their diet (Laug *et al.*, 1950). The same is true if D.D.T. is given to man (Hayes *et al.*, 1956). The animals show no untoward effect unless they are partly fasted. The D.D.T. is then released and produces its characteristic poisonous effects on the nervous system. However, extensive biochemical tests have shown that the fat tissue itself is not adversely affected even by concentrations of D.D.T. which would impair the metabolism of liver-cell constituents (Parker, 1960). If for any reason a material that had been laid down in fat was rapidly released it might produce its characteristic acute or subacute effects. Despite the obvious undesirability of allowing it to happen we cannot conclude, on such evidence as exists, that the prolonged ingestion of a material that will be laid down in fat would for this reason produce any novel toxic effect. However, no one could pretend that the evidence for this, reassuring though it may be, is anything but fragmentary.

Chemical Carcinogens

Of all the hazards that may arise from the ingestion of chemicals anywhere in our environment none creates more apprehension than the possibility that cancer may result. Work on the mechanism of carcinogenesis has received much stimulus from the study of chemical carcinogens which were themselves first recognized because of an unusual incidence of certain cancers among those in particular occupations. The skin, the respiratory tract, and the urinary tract, but hitherto neither the liver nor the alimentary tract, have been recognized as sites for chemically induced cancers in man. The liver, however, is one of the commonest sites of experimental tumours produced by chemicals in

laboratory animals. It is indeed a very common natural disease in certain parts of the world, but these do not include industrial areas. The possibility that these liver tumours in man are produced by chemical carcinogens, perhaps of plant origin, has by no means been excluded. It is often assumed that the tumours which develop in workers exposed to carcinogenic substances arise because they have ingested or absorbed small quantities of these substances over a very long time. Recent experimental work and current views of the origin of cancer make it worth re-examining this proposition. The relation of tissue injury by chemicals and the development of cancer has been reviewed by Magee (1961), on the basis of his work on liver and kidney tumours produced by a solvent that was once considered for industrial use—dimethylnitrosamine.

The theory that chronic irritation was an essential precursor of cancer, put forward with the authority of Virchow, has now largely been abandoned. That cancer develops as a two-stage process in which the chemical initiator brings about changes in or around the cells that ultimately become malignant, while the transformation itself is due to other factors, may well be true in man as it appears to be in certain experimental procedures used on animals. Indeed, "chronic irritation" might under some circumstances provide the second-stage stimulus, but it is the initial stages which must be considered in relation to the possible effects of prolonged exposure to small quantities of a carcinogen.

The somatic mutation theory implies some change brought about in the cell which is transmitted to the daughter cells. Such changes may occur in the nucleus, but it is now thought by some that visible chromosomal changes are the result rather than the cause of malignant cell growth. Chemical changes in the deoxyribonucleic acid (D.N.A.) of the nucleus can be readily brought about in the test-tube—for example, by alkylating agents—and have been shown to take place in the mouse (Brookes and Lawley, 1960) and the rat (Farber and Magee, 1960). The alkylated guanine which is present in the D.N.A. has been shown to produce chemical instability of the polymers, and it is possible that such faulty D.N.A. could lead to a cell having incomplete control of its metabolism.

The possible importance of the cell cytoplasm as a place in which carcinogenic changes may be initiated has come to the fore as a result of recent work on viruses. Cytoplasmic ribonucleic acid (R.N.A.) is also methylated by dimethylnitrosamine, and on current hypotheses such changes might initiate the development of a particle with some of the properties of a cancer-producing virus, or lead to faulty protein synthesis within the cell. This in turn could bring about enzyme deletion or immunological modifications, both of which occupy prominent places in current theories of the origin of cancer. The important common factor here is the demonstration that alkylating agents can change the composition of D.N.A. and R.N.A., and some of these alkylating agents are carcinogens. With diazomethane and N-nitroso-methyl urethane Dr. Schoental has produced tumours of the bronchus, oesophagus, stomach, and subcutaneous tissues, sometimes with single doses. Malignant tumours of the kidney will develop in rats several months or more after a single dose of dimethylnitrosamine of a size that produces a severe but reversible liver lesion but no immediate injury to the kidney (Magee and Barnes, 1959).

Another well-known carcinogen which can produce tumours in mice after single doses is urethane; the biochemical mechanisms have been discussed by Rogers (1961). On the mutation theories of cancer it has been thought that a long-term exposure to small quantities of a carcinogen would be dangerous in so far as this would increase the chances of a molecule of the carcinogenic chemical making contact with the "sensitive" constituent of the cell and so initiating the irreversible change. However, this recent work with carcinogens which are effective in single doses makes it seem at least as probable that the laws of mass action will apply in this as in other chemical reactions. Perhaps the immunological principle of optimal proportions might also determine the most effective reaction between a carcinogen and cell constituents, so as to lead to the development of a tumour. While dimethylnitrosamine will produce 100% of liver cancer at one dose level within 40 weeks, at a tenth of that dose it produced no pathological change at all in the course of 104 weeks of administration (Magee and Barnes, 1956). Thus there certainly is a safe dose of some carcinogens. However, the main point to be emphasized is the need to reconsider the way in which an exposure to a chemical carcinogen can be dangerous.

A single accident in which gross over-exposure occurs may in some cases be more dangerous than a lifetime's exposure to much smaller quantities. Clearly, under practical conditions where there was repeated exposure to a compound in small quantities more often than not the chances of gross over-exposure taking place on isolated occasions would be increased. However, if this did not happen there seems no reason to fear particularly an exposure to small quantities just because this did continue for a very long time. However, it is unlikely that all chemical carcinogens behave alike, and there is an urgent need for a great deal more experimental work in order to understand more clearly the relation of dose, duration of exposure, and other factors involved in the initiation of malignant changes in tissues by specific chemicals.

Conclusions

Intermittent or more continuous exposure to chemicals with a tremendous variety of biological properties is undergone by many people during their work in industry, their life in our cities, and even in the privacy of their homes. In some polluted atmospheres the quantities of toxic materials seem to border on those demonstrably hazardous. In others—for example, the field of food additives—most of the materials appear by current standards to be virtually harmless. In other situations, particularly where occupational exposure is concerned, the chemicals may produce some deviation from normal the significance and importance of which are at present not fully understood.

Unfortunately, there are only very limited resources available either in this country or overseas for the experimental study and proper analysis of the properties and mode of action of toxic substances. In recent years the balance of this effort has been grossly disturbed by the current clamour about the safety of chemicals used as food additives (Mulford, 1961). Increasingly greater laboratory facilities are being devoted to the carrying out of set procedures to show that substances almost biologically inert can be tolerated by rats and dogs for years on end. There are, however, still quite a number

of really poisonous materials, particularly in the environment of those in certain occupations. It is surely important to see that, in this country at least, more attention will be paid to the problems presented by such materials.

No one doubts the potential importance of irritant gases and particles in producing disease of the respiratory tract, yet are there in this country enough modern well-equipped laboratories for studying these experimentally? Few doubt the importance of cerebral activity and human behaviour, and nearly everyone is aware how easily this may be disturbed by simple things like fatigue or ill-chosen food. Many toxic chemicals have their most important effects upon the central nervous system, and some examples given above illustrate the limitations of animal experiments in detecting these, though their importance has been shown by observations on man.

Without any need to embrace the Pavlovian dogma so dear to the biologists of Eastern European countries, there seems very good reason for making a much more adequate study of the effects of certain toxic chemicals on animal behaviour as one means of detecting toxic effects at an early and, one hopes, at a reversible stage. No one will dispute the probability that a better understanding of the hazards presented by toxic chemicals in industrial or other environments will come from a more comprehensive examination of their mode of action in experimental animals. However, these findings must continually be related wherever possible to clinical observations on exposed people.

As our standards rise it is natural that the biochemist's bench or even the psychiatrist's couch will come to be regarded as being more satisfactory than the pathologist's slab as places from which to draw conclusions about the dangerous property of a chemical to which man is newly exposed. However, as the diagnostic criteria become more and more sensitive so does the problem arise of being able to say when a certain deviation from normal is something that should or should not be tolerated under the practical conditions in a modern civilization.

The need for common sense to prevail applies in this field as in any other involving human activity. At present one of the greatest needs for common sense seems to be to ensure that scientific research in the field of toxicology is applied to the situations where real hazards are known to exist.

REFERENCES

- Ahlmark, A., and Forsmann, S. (1951). *Arch. industr. Hyg.*, **3**, 386.
 Amdur, M. O. (1958-9). *Int. J. Air Pollut.*, **1**, 170.
 — Melvin, W. W., and Drinker, P. (1953). *Lancet*, **2**, 758.
 Andersson, A. (1957). *Acta med. scand.*, **157**, Suppl. 323.
 Beutler, E. (1957). *J. Lab. clin. Med.*, **49**, 84.
 — Dern, R. J., and Alving, A. S. (1955). *Ibid.*, **45**, 40.
 Brodie, B. B., Gillette, J. R., and LaDu, B. N. (1958). *Ann. Rev. Biochem.*, **27**, 427.
 — Mark, L. C., Papper, E. M., Lief, P. A., Bernstein, E., and Rovenstine, E. A. (1950). *J. Pharmacol. exp. Ther.*, **98**, 85.
 Brookes, P., and Lawley, P. D. (1960). *Biochem. J.*, **77**, 478.
 Buckell, M., and Richardson, J. D. (1950). *Brit. J. industr. Med.*, **7**, 131.
 Butler, T. C. (1949). *J. Pharmacol. exp. Ther.*, **97**, 84.
 Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S. (1956). *Science*, **124**, 484.
 Collet, A., Voisin, G. A., Daniel-Moussard, H., and Toullet, F. (1961). *Arch. environm. Hlth*, **2**, 409.
 Cremer, J. E. (1959). *Brit. J. industr. Med.*, **16**, 191.
 Dalhamn, T. (1956). *Acta physiol. scand.*, **36**, Suppl. 123.
 Doll, R. (1955). *Brit. J. industr. Med.*, **12**, 81.
 Elkins, H. B. (1961). *Arch. environm. Hlth*, **2**, 45.
 Farber, E., and Magee, P. N. (1960). *Biochem. J.*, **76**, 58P.
 Fitzhugh, O. G., Nelson, A. A., Laug, E. P., and Kunze, F. M. (1950). *Arch. industr. Hyg.*, **2**, 433.

- Gerarde, H. W. (1960). *Toxicology and Biochemistry of Aromatic Hydrocarbons*. Elsevier, Amsterdam and London.
- Grandjean, E., Münchinger, R., Turrian, V., Haas, P. A., Knoepfel, H.-K., and Rosenmund, H. (1955). *Brit. J. industr. Med.*, **12**, 131.
- Gross, P. (1960). *Arch. industr. Hlth*, **21**, 228.
- Grut, A. (1949). *Chronic Carbon Monoxide Poisoning*. Munksgaard, Copenhagen.
- Hayes, W. J., Durham, W. F., and Cueto, C. (1956). *J. Amer. med. Ass.*, **162**, 890.
- Holt, P. F. (1957). *Pneumoconiosis*. Arnold, London.
- Larizza, P., Brunetti, P., and Grignani, F. (1960). *Lancet*, **1**, 601.
- Laug, E. P., Nelson, A. A., Fitzhugh, O. G., and Kunze, F. M. (1950). *J. Pharmacol. exp. Ther.*, **98**, 268.
- Lawther, P. J. (1955). *Lancet*, **2**, 745.
- Lindgren, S. A. (1961). *Acta med. scand.*, **167**, Suppl. 356.
- Long, J. E., and Hatch, T. F. (1961). *Amer. industr. Hyg. Ass. J.*, **22**, 6.
- McCollister, D. D., Beamer, W. H., Atchison, G. T., and Spencer, H. C. (1951). *J. Pharmacol. exp. Ther.*, **102**, 112.
- Macfarland, R. A., Roughton, F. J. W., Halperin, M. H., and Niven, J. I. (1944). *J. Aviat. Med.*, **15**, 381.
- McLuen, E. F., and Fouts, J. R. (1961). *J. Pharmacol. exp. Ther.*, **131**, 7.
- Magee, P. N. (1961). *Lectures on Scientific Basis of Medicine*. Athlone Press, London. (To be published.)
- and Barnes, J. M. (1956). *Brit. J. Cancer*, **10**, 114.
- (1959). *Acta Un. int. Cancr.*, **15**, 187.
- Matzen, R. (1957). *Amer. J. Physiol.*, **190**, 84.
- Mead, J. (1961). *Physiol. Rev.*, **41**, 281.
- Mulford, K. E. (1961). *Arch. environm. Hlth*, **2**, 212.
- Nelson, N. (1961). *Symposium on Inhaled Particles and Vapours*. Pergamon Press, London.
- Parker, V. H. (1960). *Biochem. J.*, **77**, 74.
- Pattle, R. E. (1958). *Proc. roy. Soc. B*, **148**, 217.
- and Thomas, L. C. (1961). *Nature (Lond.)*, **189**, 844.
- Reid, L. (1960). *Thorax*, **15**, 132.
- Robertson, A. J., Rivers, D., Nagelschmidt, G., and Duncumb, P. (1961). *Lancet*, **1**, 1089.
- Rogers, S. (1961). *Arch. Path. (Chicago)*, **70**, 661.
- Roth, J. S., and Bukovsky, J. (1961). *J. Pharmacol. exp. Ther.*, **131**, 275.
- Russell, R. W., Watson, R. H. J., and Frankenbaeuser, M. (1961). *Scand. J. Physiol.*, **2**, 21.
- Schoental, R. (1960). *Nature (Lond.)*, **188**, 420.
- Stern, J. H., and Eisenbud, M. (1951). *Arch. industr. Hyg.*, **4**, 123.
- Stöckinger, H. E., Wagner, W. D., and Wright, P. G. (1956). *Arch. industr. Hlth*, **14**, 158.
- Surtshin, A., and Yagi, K. (1958). *Amer. J. Physiol.*, **192**, 405.
- Vigliani, E. G., and Pernis, B. (1959). *J. occup. Med.*, **1**, 319.
- Wagner, J. C., Skeggs, C. A., and Marchand, P. (1960). *Brit. J. industr. Med.*, **17**, 260.
- Williams, R. T. (1959). *Detoxication Mechanisms*, 2nd ed. Chapman and Hall, London.
- Wolfe, H. R., Walker, K. C., Elliott, J. W., and Durham, W. F. (1959). *Bull. Wld Hlth Org.*, **20**, 1.
- Zuelzer, W. D., and Apt, L. (1949). *J. Amer. med. Ass.*, **141**, 185.

"I have said that we should try to think with the whole of our past. One of the thoughts most strongly induced by this practice concerns the unevenness of progress. The most striking gains prove, on examination, to have involved serious loss. An example is alphabetic writing. This surely, one would think, is a wholly beneficent achievement of human ingenuity. It was, in fact, a necessary pre-condition of the emergence of large-scale organization, yet it wrought so decisive a change in human consciousness that it all but obliterated the memory, and certainly destroyed the understanding, of the age-old culture of pre-literate societies. The invention of the alphabet blinded men to the knowledge of some thousands of years of our human story. Much research and a great effort of the historical imagination were needed to restore to the twentieth century an appreciation of the oral cultures which writing destroyed. Yet so accomplished a stylist as Plato was near enough to the world of oral tradition to view the dissemination of the alphabet with a critical eye. He had two main fears. One, that writing would impair the memory. Has it not done so? 'Without writing,' notes Tom Harrison, 'memory is perfect, tradition exact.' Secondly, Plato felt that a book was an inadequate substitute for a living teacher. We may note that the two greatest teachers in the European tradition, Socrates and Jesus, never wrote a book. In our own educational practice at its best, books are treated only as an adjunct to the spoken word." ("Science and the Classics," Professor B. Farrington, *Nature*, September 30, 1961.)

TOBACCO SMOKING AND VENTILATORY FUNCTION OF THE LUNGS

BY

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It is a matter of everyday clinical observation that the majority of patients with chronic obstructive lung disease* give a significant history of tobacco smoking. In the minds of many this leads to the undocumented extrapolation that "smoking causes bronchitis."

A number of clinical studies have convincingly demonstrated an increased incidence of cough and sputum among smokers (see Higgins, 1959). Studies relating objective assessment of lung function to smoking habits are less susceptible to simple or uniform interpretation. At the international level (United Kingdom versus Denmark) a higher incidence of smoking in a community (U.K.) has been shown to be associated with a lower mean maximum breathing capacity (M.B.C.) (Olsen and Gilson, 1960). Smokers have been found to have a lower mean M.B.C. than non-smokers in both urban and rural areas of Great Britain (Higgins, 1957, 1959). Maximal expiratory flow rate (M.E.F.) has been shown to diminish more rapidly with increasing age in smokers than in non-smokers in a group of hospital in-patients (Flick and Paton, 1959). On the other hand, the diminution in ventilatory capacity does not run parallel with the amount of tobacco smoked (Higgins, 1959); differences between cigarette smokers and pipe smokers are sometimes more marked than differences between cigarette smokers and non-smokers (Olsen and Gilson, 1960); the scatter of ventilatory capacity among smokers as a whole is wide (Flick and Paton, 1959); and the position of ex-smokers is obscure (or even ignored) in some studies.

Sydney has a mild climate and an atmosphere that is relatively unpolluted compared with many parts of Northern Europe. Clinically, gross bacterial infection of the airways is far less common in patients with chronic bronchitis in Australia than it is in Great Britain. It was felt that a pilot study of ventilatory function in relation to smoking habits under these conditions might yield more straightforward data than earlier studies.

Methods

The subjects of the study were 302 people, aged 18 years or more, interviewed in the out-patient department of Sydney Hospital, in the following groups: (1) ambulant out-patients waiting to be seen at the gynaecology, urology, neurosurgery, general surgery, and dermatology clinics (general and special medical clinics were specifically avoided); (2) friends waiting with patients; (3) members of the medical, nursing, and lay staff associated with these clinics. On certain mornings and

*This term, recommended by a Ciba Guest Symposium (1959), is used in preference to the terms "chronic bronchitis" or "emphysema."